EVALUATION OF OXIDATIVE STRESS IN THE PATHOGENESIS OF PREECLAMPSIA BY STUDYING THE CHANGES IN PLASMA MALONDIALDEHYDE AND CERULOPLASMIN

Dissertation Submitted to

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY

in partial fulfillment of the regulations

for the award of the degree of

M.D. (BIOCHEMISTRY)
BRANCH – XIII

GOVT. STANLEY MEDICAL COLLEGE & HOSPITAL
THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY
CHENNAI, INDIA.

MARCH 2007

CERTIFICATE

This is to certify that the dissertation entitled **"EVALUATION OF OXIDATIVE STRESS IN THE PATHOGENESIS OF PREECLAMPSIA BY STUDYING THE CHANGES IN PLASMA MALONDIALDEHYDE AND CERULOPLASMIN"** is the bonafide original work of **Dr. R. MOHANDOSS** in partial fulfillment of the requirements for **M.D. (BIOCHEMISTRY) BRANCH – XIII Examination** of the Tamilnadu Dr. M.G.R. Medical University was held in March 2007.

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DECLARATION

I, **Dr. R. MOHANDOSS,** solemnly declare that the dissertation titled, "**EVALUATION OF OXIDATIVE STRESS IN THE PATHOGENESIS OF PREECLAMPSIA BY STUDYING THE CHANGES IN PLASMA MALONDIALDEHYDE AND CERULOPLASMIN"** is a bonafide work done by me at Govt. Stanley Medical College & Hospital during 2004-2007 under the guidance and supervision of **Dr. P. JAYANTHI, M.D.** Professor and Head, Department of Biochemistry, Stanley Medical College, Chennai-600 001.

The dissertation is submitted to Tamilnadu, Dr. M.G.R. Medical University, towards partial fulfillment of requirement for the award of **M.D. Degree (BRANCH – XIII) in Biochemistry.**

Place: Chennai.

Date:

(Dr. R. MOHANDOSS)

CONTENTS

ACKNOWLEDGEMENT

There is hardly any task which is more pleasant than acknowledging my gratitude to all those who have helped me in so many ways in preparing this dissertation.

I express my sincere thanks to **Dr. D.R. GUNASEKARAN, M.S. F.I.C.S** Former Dean and **Dr. T. RAVEENDRAN, M.D., D.T.C.D.,** Former Dean of Government Stanley Medical College and Hospital, Chennai – 600 001 for permitting me to use all the needed resources for this dissertation work.

My profound thanks to **Prof. Dr. P. JAYANTHI, M.D.,** Professor and Head of the department, Biochemistry Stanley Medical College for guiding me to do the dissertation and her constant inspiration given to me through out this study.

My sincere and special thanks to **Prof. Dr. RENGA RAMANI, M.D., D.G.O.,** Govt. R.S.R.M. Lying-in Hospital, Stanley Medical College for guiding me to do the dissertation.

I also thank **Prof. Dr. RAMAKRISHNAN, M.D., D.L.O.** Additional Professor of Biochemistry, M.M.C. for his valuable suggestions.

My sincere and special thanks to **Prof. CYNTHIA ALEXANDER, M.D., D.G.O.** Superintendent, Govt. R.S.R.M. Lying-in Hospital, Stanley Medical College, for permitting me to collect sample from Govt. R.S.R.M. Lying-in Hospital.

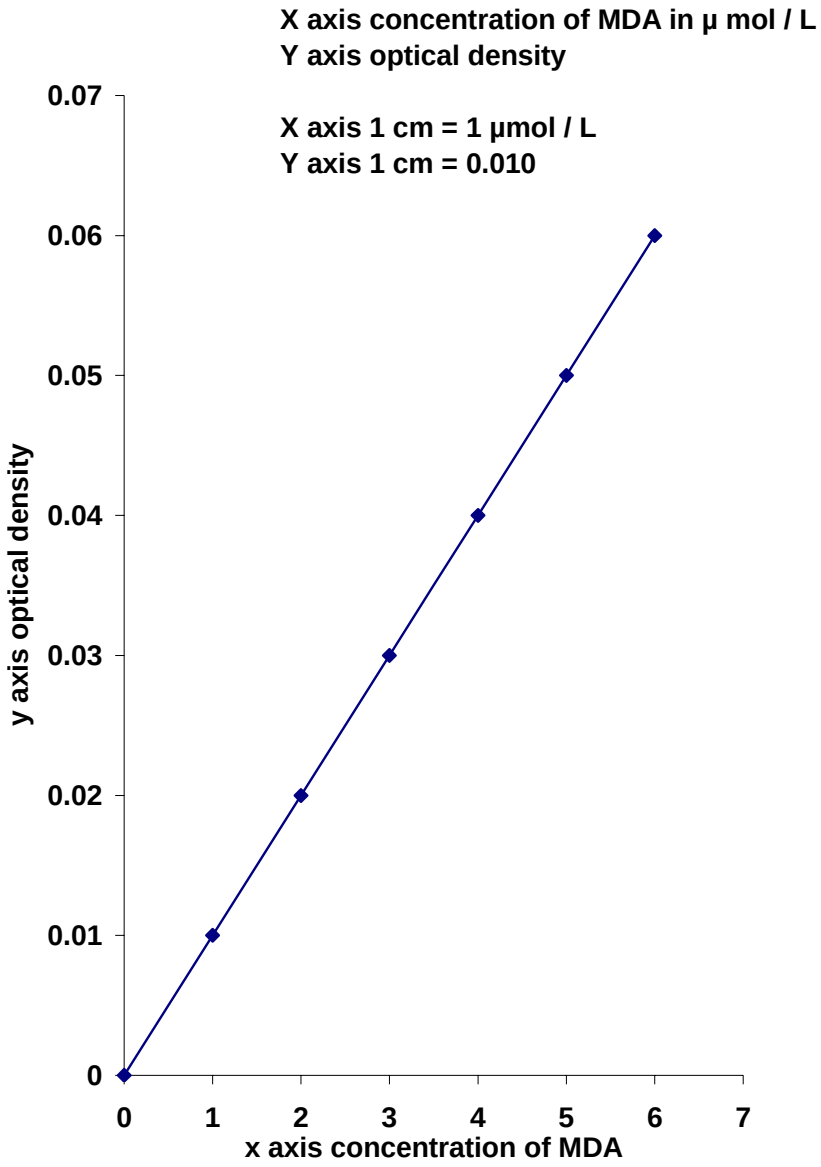
I am very grateful to assistant professors **Dr. A. VIJAYALAKSHMI M.D.,** and **Dr. R. MAHALAKSHMI D.C.H., M.D.,** Department of Biochemistry for their valuable guidance and friendly suggestion to carry out this work.

I express my thanks to Assistant Professors of Obstetrics and Gynecology department of Govt. R.S.R.M. Lying-in Hospital for helping me in case selection.

I express many thanks to all the technical staff and other staff of the department of Biochemistry, Stanley Medical College for their kind cooperation to carry out this work successfully.

I gratefully acknowledge the co-operation rendered by the patients who made it possible for this study to be carried out by me. Without their support this study would not have been possible. I salute the endurance forbearance and friendly attitude of the patients who took part in this study.

STANDARDIZATION GRAPH OF MALONDIALDEHYDE (MDA)



INTRODUCTION

Preeclampsia is a human pregnancy specific, multi system disorder that is characterized by hypertension and proteinuria[1]. Inspite of intensive effort to identify the pathophysiological mechanism neither a specific cause nor a pathogenesis has been identified.

Roes, EM et al.hypothesised that endothelial dysfunction plays a major role in preeclampsia[2].

Carl A. Hubel reviewed the role of oxidative stress in vascular endothelial cell damage and also recorded increased level of lipid peroxidation products in preeclampsia[3].

Atamer Y et al. reported that the plasma antioxidant activity in preeclampsia is defective, and could be the causative factor that is responsible for endothelial damage[4].

Gutteridge JMC et al. reported that Ceruloplasmin is the major plasma antioxidant and its activity is found to decrease in preeclampsia[5]. The increased lipid peroxidation along with decreased ceruloplasmin activity may play a role in pathogenesis of preeclampsia.[6]

The present study was carried out to determine the level of plasma, malondialdehyde [MDA] as a marker of lipidperoxidation and ceruloplasmin as an antioxidant against lipid peroxidation. By performing the above parameters in preeclampsia the antioxidant status and oxidative stress were studied.

REVIEW OF LITERATURE

Southern, PA et al. reviewed the involvement of free radicals and other reactive species in the pathogenesis of different human diseases[7].

Seis. H. in 1991 defined oxidative stress as a disturbance in prooxidant - antioxidant balance in favour of the former leading to potential tissue damage[8].

Carl. A. Hubel reported that the vascular endothelial changes in preeclampsia result from oxidative stress.

Roberts, JM et al. proposed that the products of feto placental unit enter the circulation and then initiate maternal patho physiological changes[9].

Walsh, SC et al. reported that lipid peroxidation products primarily as thiobarbituric acid reactive substances (which include malondialdehyde) are increased in plasma of women with preeclampsia[10].

Lipid hydroperoxides regulate enzymes and redox sensitive genes[11]. However uncontrolled lipid peroxidation can result in cellular dysfunction and damage.

Under normal conditions antioxidant defence system in the body inhibits lipid peroxidation by interfering with the chain reaction of peroxidation.

The major intracellular antioxidants are catalase, superoxide dismutase, and glutathione peroxidase. But the extra cellular fluid plasma contains little or no catalase activity and only low activities of superoxide dismutase and

glutathione peroxidase. Transferrin and ceruloplasmin are shown to be major plasma antioxidants[12].

HYPERTENSIVE DISORDERS COMPLICATING PREGNANCY

The term hypertensive disorders complicating pregnancy includes a heterogenous collection of hypertensive disorders which are peculiar to pregnancy. Nearly 18% of maternal deaths are related to these hypertensive disorders.

The working group of National High Blood Pressure Education programme proposed a classification for the hypertensive disorders complicating pregnancy[13].

There are five types of hypertensive disorders complicating pregnancy.

They are

1) Gestational hypertension

2) Preeclampsia

3) Eclampsia

4) Preeclampsia super imposed on chronic hypertension

5) Chronic hypertension

DIAGNOSIS OF HYPERTENSIVE DISORDERS COMPLICATING PREGNANCY

The criteria for diagnosing hypertensive disorders complicating pregnancy are outlined by National High Blood Pressure Education Programme Working group[13].

1) Gestational Hypertension

BP ≥ 140 /90 mmHg for the first time during pregnancy

No proteinuria

BP returns to normal after 12 weeks of postpartum

2) Preeclampsia

Minimum criteria for diagnosing preeclampsia are

1) BP ≥ 140 / 90 mmHg after 20 weeks of gestation.

2) Proteinuria ≥ 300 mg / 24 hours or $\underline{\geq}$ 1 + by dipstick method.

Presence of the following signs or symptoms increased the certainty of preeclampsia

BP ≥ 160 / 110 mm Hg

Proteinuria 2.0 g / 24 hours or $\underline{\geq}$ 2 + by dipstick method

S. creatinine > 1.2 mg / dl unless known to be previously elevated.

Microangiopathic haemolysis as evidenced by increased LDH.

Elevated Alanine & Aspartate transaminase.

Persistent headache or other cerebral or visual disturbance

Persistent epigastric pain

Eclampsia

Occurrence of seizures that cannot be attributed to any other cause in women with preeclampsia.

Preeclampsia Superimposed upon Chronic Hypertension

New onset of proteinuria ≥ 300 mg/ 24 hrs in hypertensive women but no proteinuria before 20 weeks gestation.

A sudden increase in proteinuria or blood pressure or platlet count < 100,000 / mm^3 in women with hypertension and proteinuria before 20 weeks of gestation.

Chronic Hypertension

BP ≥ 140 / 90 mmHg before pregnancy or diagnosed before 20 weeks gestation or hypertension first diagnosed after 20 weeks gestation and persistent after 12 weeks postpartum.

PRE-ECLAMPSIA

Preeclampsia is a pregnancy specific syndrome of reduced organ perfusion secondary to vasospasm and endothelial activation.

Incidence & Risk Factors

The incidence varies from 8-10 percent in Indian population.

1. It occurs chiefly in primigravidae[14].
2. It is more common in multiple pregnancy, hydramnios and vesicular moles.
3. It may occur in association with maternal disease like diabetes mellitus, hypertension and renal disorder[14].

Diagnosis

The minimum criteria for diagnosing preeclampsia are hypertension and proteinuria[15].

Proteinuria is described as the presence of 300mg or more of urinary protein per 24 hours or persistent 30 mg / dL (1 + dipstick) in random urinary sample.

Hypertension is diagnosed when blood pressure is 140/90 mmHg or more using Karotkoffs phase V to define diastolic pressure[16].

Signs & Symptoms

Apart from proteinuria and hypertension mild preeclampisa have no other signs or symptoms. In severe cases symptoms of headache, restlessness, vomiting, epigastric pain, and visual disturbance may be present[17].

Abnormal biochemical findings in renal, hepatic, hematological functions may also be present in preeclampsia.

The abnormal biochemical findings include

a) Platelet count < 100, 000 / mm^3

b) Elevated serum uric acid more than 5.9 mg / dl.

PATHOLOGY

Most of the pathological consequences are due to vasospasm that leads to decreased end organ perfusion. Preeclampsia adversely affects the mother and fetus.

Changes in the maternal organs and functions consequent to preeclampsia

1) Cardiovascular Changes

Benedetti and their associates reported high systemic vascular resistance, hyperdynamic ventricular function, and reduced blood volume in preeclampsia[18].

2) Hematological Changes

Thrombocytopenia is the most common finding in preeclampsia[19]. It is likely to result from platelet activation and consumption. Thrombocytopenia is some time accompanied by erythrocyte destruction. This destruction is evidenced by the presence of reticulocytosis.

Endocrine Changes

In preeclampsia there is sodium retention and hypertension. Both of these factors lead to decrease secretion of renin by juxtaglomerular apparatus. Renin is necessary for the conversion of angiotensinogen to angiotensin I. Angiotensin I is then converted to Angiotensin II by Angiotensin converting enzyme. Angiotensin II stimulates aldosterone secretion. Due to decrease renin secretion the aldosterone secretion also impaired[20].

Fluid Changes

In preeclampsia renal endothelial injury leads to proteinuria.

Renal Changes

Glomeruli are enlarged with glomerular capillary endothelial swelling. The swollen endothelial cells block the capillary lumen[21].

Renal perfusion and GFR are reduced. Preeclampsia is associated with increased concentration of serum creatinine and uric acid and diminished urinary calcium excretion[22].

Liver

Due to numerous scattered areas of subscapular haemorrhage its appearance is mottled. Increased liver enzymes are due to periportal haemorrhagic necrosis in the periphery of liver lobules[23].

Placenta

Placenta plays a major role in maternal endothelial dysfunction in preeclampsia[24]. This is evidenced by the disappearance or remission of preeclampsia after termination of pregnancy. The genesis of preeclampsia is clearly related to deficient trophoblast invasion and failure of uterine artery remodeling[25]. Preeclampsia placentas show abnormal expression of integrin molecules. Integrin molecules regulate cell to cell and cell to matrix interactions. As a result trophoblastic invasion is inhibited and spiral artery remodeling is often limited to the decidual portions. So the myometrial segments do not widen and remain contractile. This defective spiral artery remodeling results in reduced utero placental perfusion and foci of placental hypoxia or ischemia.

Numerous markers of oxidative stress are altered in placenta of preeclampsia.

Anatomically the placenta of preeclampsia shows increased syncytial knots, areas of infarcts[26]. Microscopically there is a loss of syncytium, proliferation of cytotrophoblast, villous necrosis, and fibrinoid degeneration of maternal decidual arteries.

PATHOPHYSIOLOGY

Vasospasm: Vasospasm is basic to pathophysiology of preeclampsia. Vasoconstriction accounts for the development of arterial hypertension[27].

Increased pressure response

There is increased sodium retention in preeclampsia. The shift of sodium into arterial wall increases the sensitivity of vascular endothelium to circulating vasopressors[28].

Prostaglandins

Thromboxane A2 is increased; prostacylin and prostaglandin E2 are decreased in preeclampsia, resulting in vasoconstriction[29].

Vascular Endothelial Growth Factor (VEGF)

It is a glycoprotein and selectively mitogenic to vascular endothelium. It is important for vasculogenesis and micro vascular permeability. VEGF has been reported to increase in preeclampsia. Increased VEGF may represent a compensatory mechanism attempting to restore utero placental blood flow to normal[30].

Oxidative Stress

Lorentzen, B. et. al. reported that lipid alteration may promote oxidative stress in preeclampsia[31].

The mean TGL and FFA concentrations undergo near doubling in women with preeclampsia relative to normal pregnancy. The plasma TGL level rises above 400mg / dl[32]. This hyper triglyceridemia of preeclampsia is also accompanied by increased prevalence of smaller and denser LDL particles and decreased HDL cholesterol. Smaller denser particles of LDL are more atherogenic. Due to their small size, these particles more readily infiltrate into arterial tissue, the presumed site of LDL oxidation. Compared to large particles of LDL, the small dense LDL particles are more susceptible to oxidation[33].

In trophoblasts and macrophages of the normal placenta, scavenger receptor activity greatly exceeds native LDL receptor activity. LDL might be prone to oxidation during its relatively slow travel through the intervillous space in direct contact with trophoblast cells[34]. The smaller and denser LDL particles formed during pregnancy are increasingly susceptible to oxidation. The stable peroxidation metabolites produced during placental oxidative stress enter the maternal circulation and produce widespread endothelial damage and dysfunction[35].

FREE RADICAL

STRUCTURE OF ATOM

An atom is a simplest unit of an element. It is made up of a central nucleus which contains protons and neutrons. The protons are positively charged and the neutrons are uncharged. The nucleus is surrounded by electrons. The electrons are negatively charged. Stable compounds have even number of electrons paired in outer most orbital with opposite spin. Barrywell defined free radical as "any species capable of independent existence that contain one or more unpaired electron. An unpaired electron is one that occupies an atomic or a molecular orbital without a pair[36].

Free radicals include oxygen centered radicals, carbon centered radicals, nitrogen centered radicals and transition metals.

Role of Free Radicals in Lipid Peroxidation

In lipid peroxidation oxygen centered radicals initiate and propagate the chain reaction of peroxidation. The transition metals decompose hydro peroxides, the end product of lipid peroxidation.

Oxygen Centered radicals

The oxygen centered radicals include

1. Hydroxyl radicals $OH^{\bullet}$
2. Peroxyl $RO_2^{\bullet}$

3. Alkoxyl $RO^{\bullet}$

4. Super oxide $O_2^{\bullet -}$

Among the oxygen centered radicals $OH^{\bullet}$ radicals initiate lipid peroxidation[37], alkoxyl ($RO^{\bullet}$) and peroxyl ($ROO^{\bullet}$) radicals propagate the chain reaction.

HYDROXYL RADICALS :

Among the oxygen centered radicals, hydroxyl radicals are more reactive.

Formation of $OH^{\bullet}$ radicals

1. Absorption of radiation energy: Ionizing radiation produced by ultraviolet and X rays can hydrolyze water into hydroxyl ions[38].

2. During oxidative phosphorylation oxygen undergoes reduction to produce water. Partial reduction of oxygen leads to the formation of oxygen free radicals including hydroxyl radical.

3. Fenton reaction: Ferrous ion has 4 unpaired electron in 3'd' orbital.

These ions interact with hydrogen peroxide and form hydroxyl radical.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH^{-}$$ [39]

ROLE OF TRANSITION METALS IN LIPID PEROXIDATION

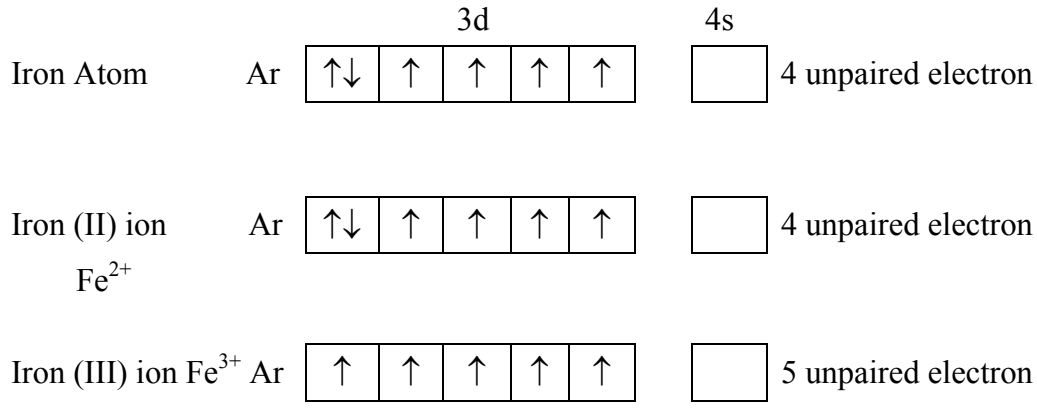
Many metals in the Ist row of'd' block in the periodic table contain unpaired electron and can qualify as free radical.

Iron and copper are the two metals which take part in lipid peroxidation.

Iron In Lipid Peroxidation

Iron has 2 common oxidation numbers. Iron atom has 4 unpaired electrons. Ferrous iron has 4 unpaired, and Ferric iron has 5 unpaired electrons.

The electron configuration of iron radicals



Iron bound to phosphate esters, carbohydrates, membrane lipids, can decompose lipid peroxides to form alkoxyl and peroxyl radicals.

Role of iron in peroxide decomposition

Lipid peroxides are usually stable. They decompose in the presence of metal ions[40].

Ferrous ion combines with lipid peroxides and produce alkoxyl radical.

$$ROOH + Fe^{2+} \rightarrow Fe^{3+} + RO^{\bullet}$$

Alkoxyl radical can abstract hydrogen atom from a second molecule of PUFA and forms peroxyl and carbon centered radical .

$$RO^{\bullet} + RH \rightarrow ROH + R^{\bullet}$$

$$ROOH + RO^{\bullet} \rightarrow ROO^{\bullet} + ROH$$

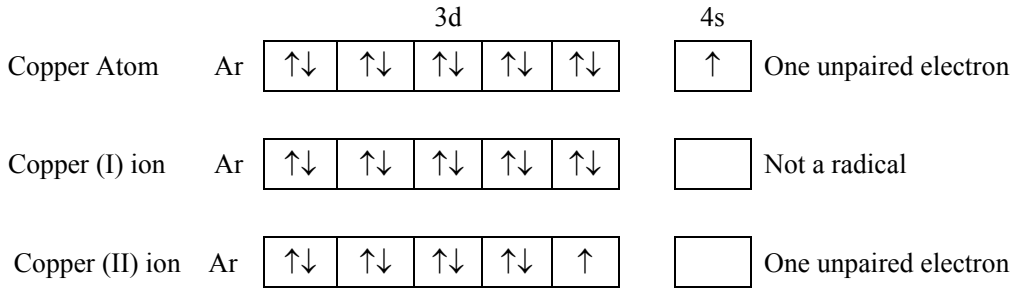
Ferric ions combine with lipid hydro peroxides and decompose them to peroxyl radicals[40].

$$ROOH + Fe^{3+} \rightarrow ROO^{\bullet} + H^{+} + Fe^{2+}$$

Role of Copper in decomposition of lipidperoxides

Copper has two oxidation numbers Cu^{+} (cuprous) Cu^{2+} (cupric). Copper atom has one unpaired electron, and cuprous ion has one unpaired electron.

The electron configurations are as follows.



They act mainly on preformed lipid hydro peroxides[41].

Cupric ion decomposes the peroxides to peroxyl radical.

$$ROOH + Cu^{2+} \rightarrow Cu^{+} + RO_2^{\bullet} + H^{+}$$

Cuprous ion decomposes peroxides to alkoxyl radical.

$$ROOH + Cu^{+} \rightarrow RO^{\bullet} + Cu^{2+} + OH^{-}$$

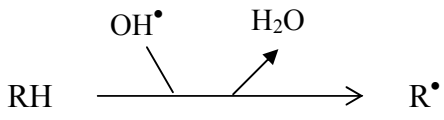
Both the peroxyl and alkoxyl radicals promote the propagation of lipid peroxidation.

LIPID PEROXIDATION

Lipid peroxidation is defined as oxidative deterioration of lipids. Poly unsaturated fatty acids (PUFA) are more susceptible to lipid peroxidation. This reaction proceeds in a self amplifying manner. So it is called chain reaction. This chain reaction occurs in three stages. They are (1) Initiation (2) Propagation (3) Termination.

INITIATION

Any radical that is capable of abstracting a hydrogen atom from a methylene group of PUFA can initiate peroxidation. More frequently $OH^{\bullet}$ radical initiate lipid peroxidation by abstracting $H^{\bullet}$ [37]from methylene group. This leaves an unpaired electron on the methylene carbon. So a carbon centered radical [$R^{\bullet}$] is generated.



PROPOGATION

The carbon centered radical can undergo various reactions. They are:

1. Two of the carbon centered radicals collide within the membrane and they cross link adjacent fatty acid side chains[42].

2. Under aerobic condition the carbon centered radical combines with oxygen and gives peroxyl ($ROO^{\bullet}$) radical.

$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet}$$

This peroxyl radical attacks adjacent fatty acid side chain to form carbon centered radical

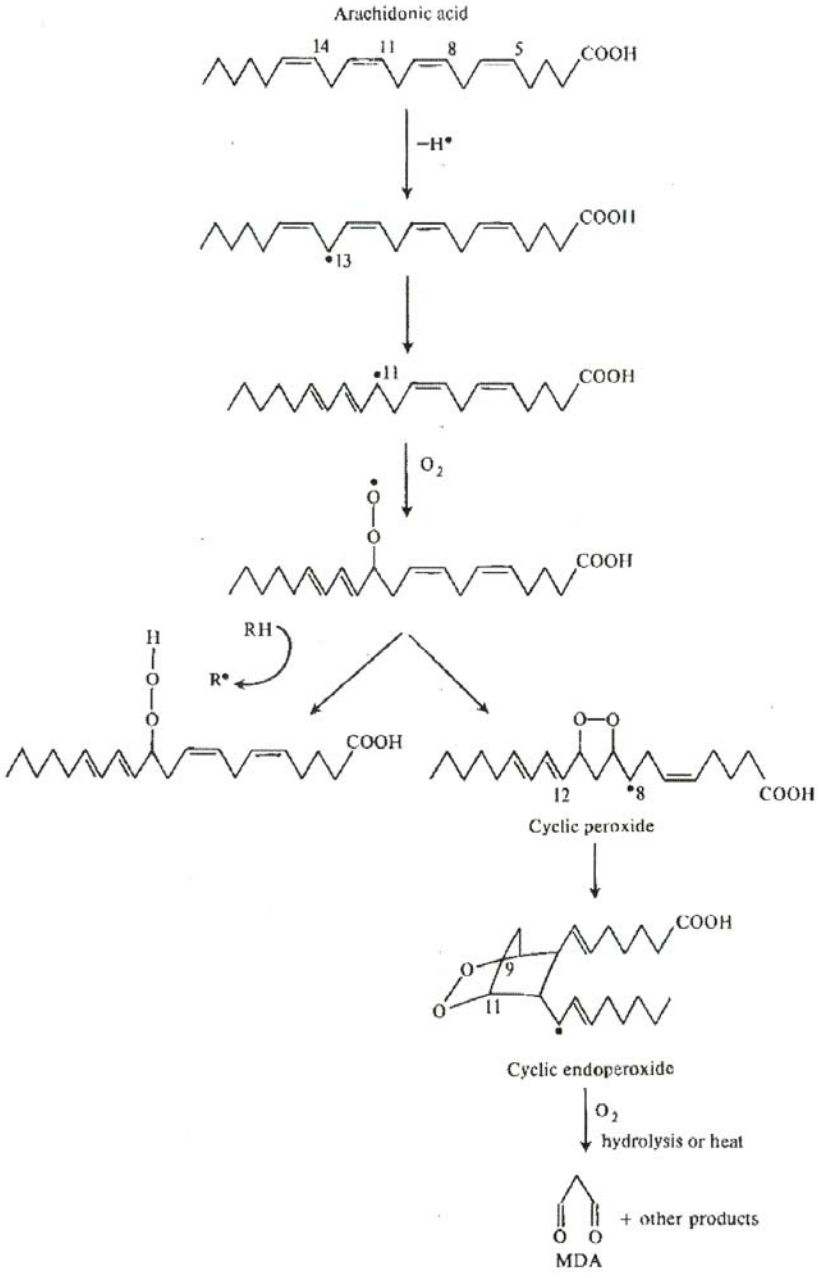
$$ROO^{\bullet} + RH \rightarrow ROOH + R^{\bullet}$$

The carbon radical formed can react with oxygen to form another peroxyl radical. In this way the chain reaction of lipid peroxidation continues[42].

The peroxyl radical combines with the hydrogen atom that it abstracts from PUFA to give a lipid hydroperoxide.

Peroxyl radical can attack a double bond in the same side chain of PUFA to produce cyclic peroxide and cyclic endoperoxides. Hydrolysis of cyclic endoperoxides produce Malondialdehyde[43].

LIPID PEROXIDATION AND FORMATION OF MALONDIALDEHYDE



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BIOLOGICAL EFFECTS OF THE PRODUCTS OF LIPID PEROXIDATION:

During the propagation of lipid peroxidation the peroxyl and alkoxyl radicals are generated. The end product lipid hydroperoxide undergoes decomposition and produces malondialdehyde, hydroxy nonenals (HNE) and isoprostanes[45]. These products serve as a marker for lipid peroxidation and are implicated in damages caused to various cellular structure and function.

EFFECTS OF MALONDIALDEHYDE

MDA attacks various amino acid residues of protein and form inter and intra molecular cross links. MDA reacts with guanine of DNA and can introduce mutagenic lesions[46].

HYDROXY NONENAL [HNE]

HNE generated in vivo forms adduct with protein and DNA. HNE inhibits cell growth and it has chemotactic activity. It can modify lipoproteins and promote atherosclerosis[47].

EFFECTS OF ALKOXYL AND PEROXYL RADICALS

The peroxyl and alkoxyl radicals of lipid peroxidation can cause severe damage to membrane proteins. These radicals can damage specific amino acid residue present in various proteins[48].

Proteins contain histidine residues at their metal binding site. Modification of these residues produce signal sequences that are recognized by proteases. The proteases degrade such proteins[49].

Cell membranes contain surface receptors for hormones and cytokines. The alkoxyl and peroxyl radicals damage the protein component of these receptors.

Enzymes like glucose-6-phosphatase, glycerol-3-phosphate acyl transferase are inactivated by alkoxyl and peroxyl radicals.

Various protein channels and pumps necessary to maintain intracellular ion balance are damaged by lipid peroxides[50]. The Ca^{2+} ATpase of endoplasmic reticulum, Na,K^{+-} ATpase, and K^{+} channels can also be damaged during lipid peroxidation.

As lipid peroxidation decreases the membrane fluidity and increases the permeability of the bilayer, substances that usually can not cross the membrane enter the cells and destroy them. Rupture of lysosomal membrane can also results in the release of hydrolytic enzymes into the cell. These enzymes damage the other intracellular structures.

EFFECTS OF ISOPROSTANES

One of the isoprostane 8-epi.$PGF_{2\alpha}$ is a potent vasoconstrictor and platelet aggregator[51].

MALONDIALDEHYDE

Malondialdehyde [MDA] is one of the low molecular weight end products of lipid hydroperoxide decomposition and most often measured as an index of lipid peroxidation. MDA can also be formed during eicosanoid metabolism.

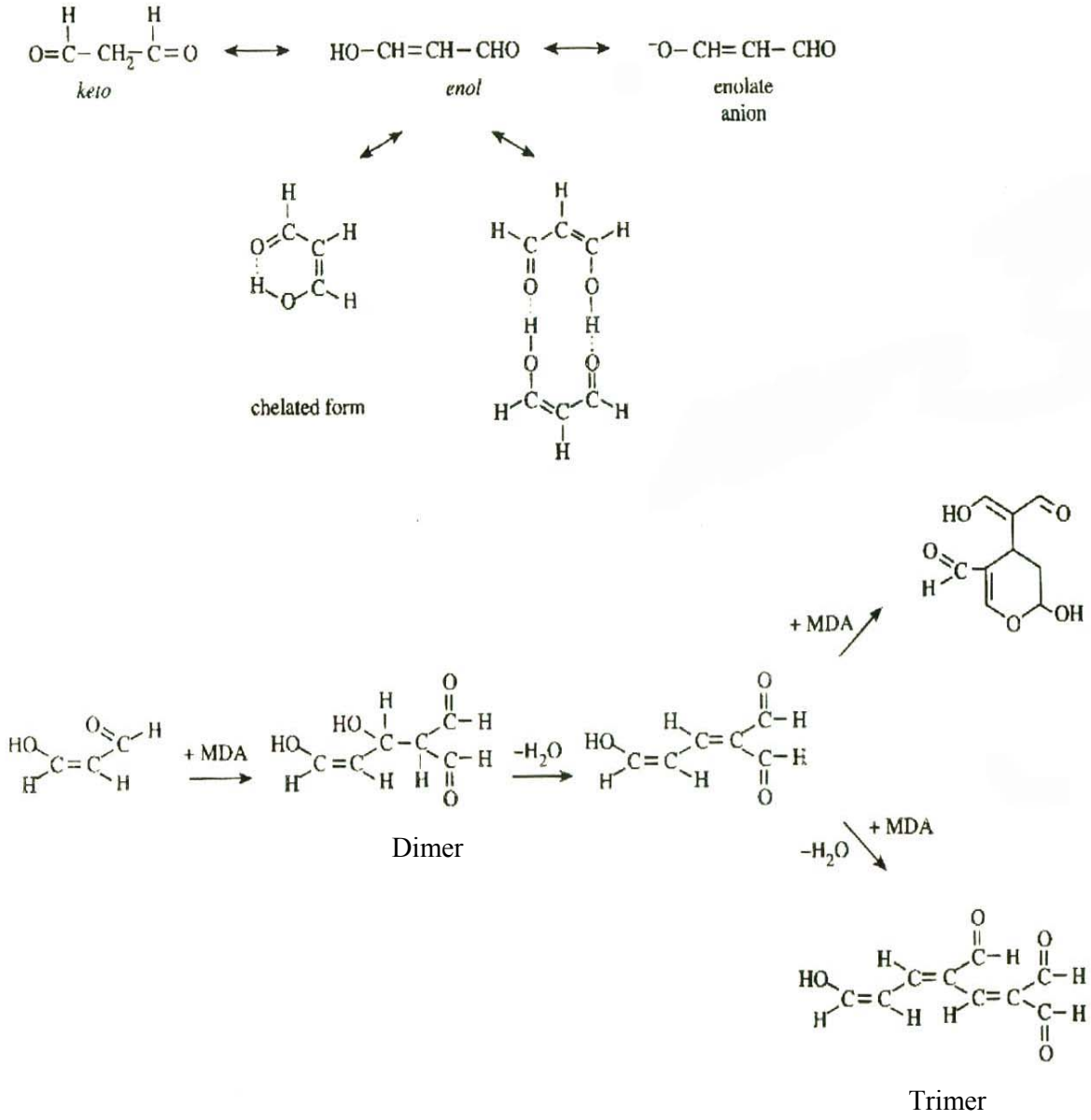
STRUCTURE OF MDA

$O = CH - CH_2 - CH = 0$

MDA exists in various form depending on pH[52]. At physiologic pH it exists as the enolate anion. In acidic pH it exists as undissociated enol and keto forms. Like most aldehydes MDA in aqueous solution is prone to aldol condensation which produce dimmers, trimers and large polymers.

MDA is rapidly metabolized in mammalian tissue. Aldehyde dehydrogenase oxidize MDA to malonic acid. Malonic acid is decorboxylated to acetaldehyde. Acetaldehyde is oxidized to acetate by aldehyde dehydrogenase

.**Various forms of MDA in aqueous solution**



Reproduced from '**Free Radicals in Biology and Medicine'** by Barry Halliwell and John M.c. Gutteridge.[53]

ANTI OXIDANT DEFENCE SYSTEM

Halliwell et al., defined antioxidant as "any substance when present at low concentrations compared with those of an oxidizable substrate significantly delays or prevents oxidation of substrate".[54]

The major intracellular antioxidants are catalase, superoxide dismutase and glutathione. The plasma antioxidants are Vitamin C, Vitamin E, and Ceruloplasmin.

Antioxidants effective against lipid per oxidations :

The hydroxyl radicals are effectively scavenged by glutathione. The peroxyl and alkoxyl radicals are detoxified by vitamin C and E. Ceruloplasmin prevents copper and iron induced decomposition of lipid hydroperoxides.

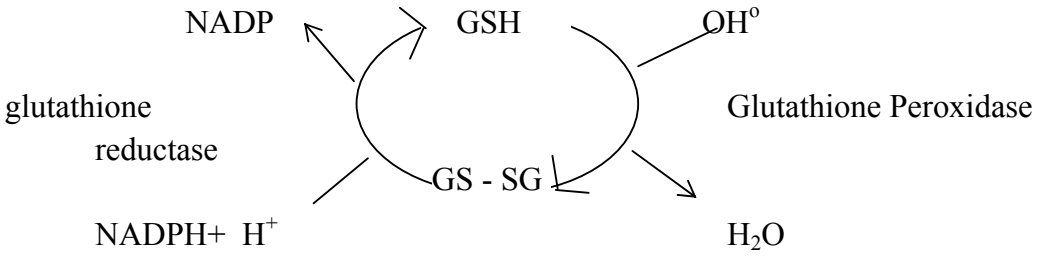
Antioxidant role of Glutathione

Glutathione is γ glutamyl cysteinglycine. It is present in reduced state where glutamate is linked to cystein and glycine.

Glutathione detoxifies hydroxyl radicals. This reaction is catalyzed by selenium containing glutathione peroxidase. During this reaction glutathione is oxidized to glutathione disulfide (GS-SG)[55].

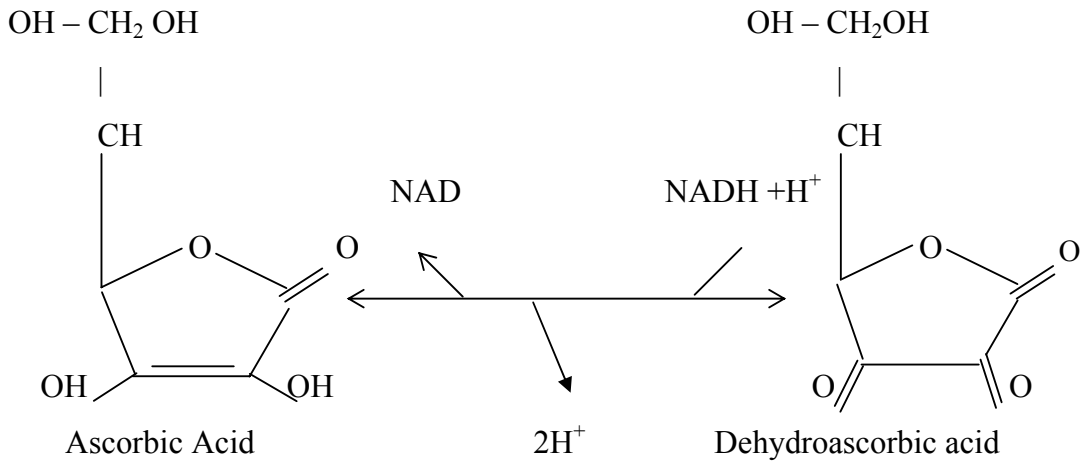
The cell regenerates reduced glutathione by a reaction catalyzed by glutathione reductase. This enzyme utilizes NADPH as a source for electrons. The NADPH is generated from oxidative phase of HMP shunt.

Mechanism of hydroxyl radical scavenging by glutathione



ASCORBIC ACID

Ascorbic acid (Vitamin C) is a water soluble vitamin. It is the enolic form of an α ketolactone. The molecular structure contains two ionizable OH group. Its antioxidant properties are due to its ability to donate electrons from the OH group[56]. It is an effective scavenger of many free radicals mainly peroxyl radicals. During this process it losses two electrons and is converted first to ascorbyl radical and then to dehydroascorbate[57]. It protects lipoproteins from peroxidation by scavenging peroxyl radicals before they propagate lipid peroxidation.



The enzyme dehydroascorbate reductase converts dehydroascorbic acid to ascorbic acid using NADH as reductant.

Apart from peroxyl radicals vitamin C scavenges hydroxyl and super oxide radicals. vitamin C plays a major role in regenerating active form of vitamin E[58].

Vitamin E

Vitamin E is a lipid soluble vitamin. It is the most powerful inhibitor of propagation of lipid peroxidation. Vitamin E has hydroxylated aromatic ring (chromanol ring) and isoprenoid side chain. The chromanol ring contains a phenolic hydroxyl group. Vitamin E is highly lipophilic and resides almost exclusively in cell membrane. In the lipid bilayer the chromanol ring lies at the surface, and the side chain is inserted into the bilayer[59].

During the propagation stage of lipid peroxidation the peroxyl radicals attack the second molecule of PUFA and produce carbon centred radical.

The carbon centred radical under aerobic condition combines with oxygen and again produce peroxyl radical. This peroxyl radical propagates the chain reaction of lipid peroxidation.

Vitamin E prevents the propagation of lipid peroxidation induced by peroxyl radical. The hydroxyl group present on the chromanol ring of Vitamin E reacts with peroxyl radical, to form the corresponding hydroperoxide and tocopheroxyl radical (Vitamin E - $O^{\bullet}$)[60]. In this way Vitamin E acts as a chain breaking antioxidant preventing further autooxidation of lipids.

In the absence of Vitamin E

$$ROO^{\bullet} + RH \rightarrow ROOH + R^{\bullet}$$

$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet}$$ thus more free radicals are produced.

In the presence of Vitamin E

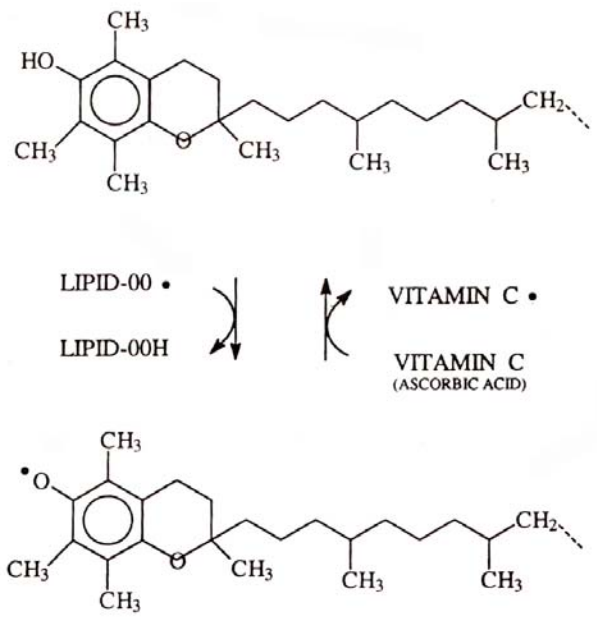
$$ROO^{\bullet} + \text{Vit E} - OH \rightarrow ROOH + \text{Vit E} - O^{\bullet}$$

here tocopheroxyl radical is produced[60].

ANTIOXIDANT NET WORK

The tocopheroxyl radical Vit E – $O^{\bullet}$ is not the active form of Vitamin E. Vitamin E is converted to tocopheroxyl radical while scavenging peroxyl radicals. When tocopheroxyl radical emerges from lipid bilayer it accepts an electron from ascorbic acid and returns to its reduced state Vitamin E. During this process ascorbic acid loses one electron and becomes ascorbyl radical. In this way Vitamin E necessary for free radical scavenging is continuously regenerated[61].

Tocopherol



Tocopheroxyl radical

Oxidation of Vitamin E by lipid radicals and reduction by Vitamin C.

Reproduced from '**Modern Nutrition in Health and Disease'[62] by Maurice E Shills.**

CERULOPLASMIN

BIOCHEMISTRY

It is an α 2 globulin which has single polypeptide chain with 1046 amino acids and three glucosamine linked oligosaccharide side chain. The molecular mass is about 132 KD. Each molecule contains 6-8 Copper atoms most of which are tightly bound.

SYNTHESIS

It is synthesized primarily in hepatic parenchymal cells. The peptide chain is formed first then Copper is added[63]. The CHO side chains are subsequently added in the endoplasmic reticulum. Copper appears to be essential for the normal folding of polypeptide chain and possibly for normal CHO side chain attachment.

REMOVAL

Sialic acid is slowly removed from circulating ceruloplasmin by tissue and plasma neuroaminidase, resulting in exposure of the terminal galactose residue on carbohydrate side chain. Once a critical number of galactose residues are exposed the protein is rapidly removed by galactose receptors of hepatic parenchymal cell and catabolized.

Function

It can function either as an antioxidant or antioxidant depending on the other factors such as the presence of free ferric ions and ferritin binding site.

Acting as ferroxidase, ceruloplasmin is vitally important in regulating the ionic state of iron in particular oxidizing $Fe3^{+}$ to $Fe2^{+}$. It thus permits the incorporation of iron into transferrin without the formation of toxic iron products[64].

Under physiological condition ceruloplasmin is also important in the control of membrane lipid oxidation – probably by direct oxidation of cations thus preventing their catalysis of lipid peroxidation.

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Ceruloplasmin inhibits the decomposition of lipid hydroperoxides exhibited by copper ions by binding to the metal.

AIM OF THE STUDY

The aim of the study was to evaluate the role of oxidative stress in the pathogenesis of preeclampsia by measuring the plasma MDA and CP as a markers for oxidative stress in preeclampsia. The study includes the measurement of ceruloplasmin activity to assess the decreased antioxidant capacity in preeclampsia. The relationship between MDA and CP were statistically analysed to assess the oxidative stress in preeclampsia.

MATERIALS & METHODS

STUDY DESIGN

The study was conducted after obtaining approval from ethical committee of Stanley Medical College.

The study population includes three different groups.

Group 1

Consists 30 non pregnant, normotensive females.

Age 21 – 32

Parities $P_{0,}P_{1,}P_{2.}$

Group 2

Consists 30 normotensive pregnancies.

Age group between 21 - 32, Gestational age 20 - 35 weeks and obstetric score $G_1 P_0$, $G_2 P_1$, $G_3 P_2$.

Group 3

Consists 30 preeclampsic pregnancies.

Age group between 21 - 32, Gestational age 20 - 35 weeks and obstetric score $G_1 P_0$, $G_2 P_1$, $G_3 P_2$.

Group 1 served as control for group 2.

Group 2 served as control for group 3.

Normal pregnant and preeclampsic women were chosen from G.R.S.R.M. lying in hospital. They were included into the study after obtaining informed consent. Healthy unpregnant subjects were chosen from general population and after obtaining informed consent they were included into the study.

Diagnosis of Preeclampsia

The diagnosis of preeclampsia was made on the basis of elevated blood pressure and proteinuria.

Blood Pressure

Hypertension was diagnosed when blood pressure was $\geq$ 140 / 90 mmHg, in a pregnant woman after 20 weeks of gestation. The Korotkoffs Phase V was used to determine diastolic blood pressure.

Proteinuria

The degree of proteinuria was detected by dipstick method.

A dipstick reading $\geq$1+ in a random sample was considered as proteinuria.

Case Selection

Inclusion Criteria: Group 1 :

Non pregnant, normotensive women with age between 21 - 32 years, without hyper tension and proteinuria.

Group 2

Pregnant women with age group 21 - 32 years without hypertension and proteinuria with gestational age 20 - 35 weeks.

Group 3

Proven cases of preeclampsia with age group of 21 - 32 years with gestational age of 20 - 35 weeks.

Exclusion Criteria

1. History of hypertension, diabetes mellitus, tuberculosis, cardio vascular disorder.
2. Bad obstetric history.
3. Primary lipid disorder.
4. Any infection.
5. Anemia.

Sample Collection

After obtaining written and informed consent urine and blood samples were collected.

Collection of Blood Samples

5 ml of blood was collected from each subject participating in this study. The samples were collected in a EDTA tubes. The tubes were allowed to stand for 15 minutes and then centrifuged. The plasma was separated and MDA and CP analysis were performed immediately.

Collection of Urine Sample

Random mid-stream urine was collected in a test tube and the degree of proteinuria was estimated immediately.

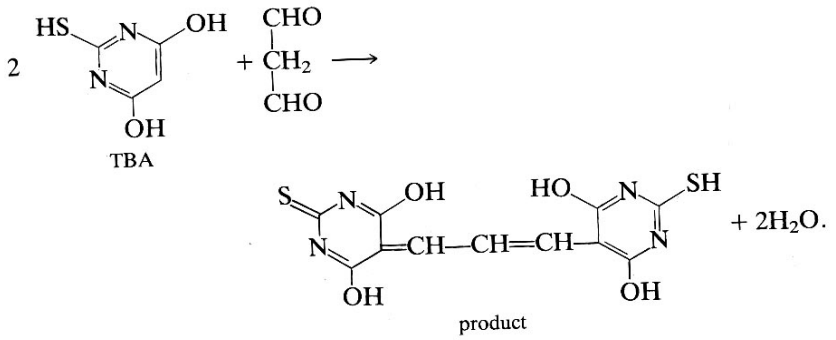
Source of Chemicals

All the chemicals used in the study were bought from HIMEDIA chemicals.

The dip sticks used for determination of proteinuria were bought from BIOLAB diagnostics.

STANDARDIZATION OF MALONDIALDEHYDE BY BEUJE JA & AUSTIN JD METHOD[65].

Malondialdehyde reacts with thiobarbituric acid forming MDA - TBA2 adduct with pale pink colour that absorbs light strongly at 532 nm.



Reagents

Thiobarbituric acid : 26 mmol / L : Molecular weight of thiobarbituric acid is 144.15. 3747 mg was taken in a volumetric flask. To this deionised water was added slowly with continuous stirring. After it was completely dissolved the volume was made to 1000 ml by adding deionised water.

Trichloroacetic Acid (0.92 mol / L)

153.318 g of TCA was taken in a volumetric flask and dissolved in **0.25** mol/L of HCL.The final volume was made to **1000**ml by adding **0.25** mol/L of HCL

Preparation of Standards

TMP (1, 1, 3,3, Tetramethoxy propane) was used to prepared standards for malondialdehyde.

The molecular weight of TMP is 164.2.

Preparation of Stock 1 : 164.2 mg of TMP was taken in a 1000 ml volumetric flask, and made to 1000 ml by adding deionised water. This corresponds to 1mmoL/L or 1000 µmol/L

Stock 2 : 10 ml of stock 1 was mixed with 90 ml of deionised water. This corresponds to 100 µmol/L

Working standards

From stock 2 various concentration of working standards were prepared as follows.

Preparation of various concentration of MDA Standards

Standard Concentration MDA in µmol / L	Volume of Stock 2 in ml	Volume of distilled water in ml
1	.1 ml	9.9 ml
2	.2 m1	9.8 ml
3	.3 ml	9.7 ml
4	.4 ml	9.6 ml
5	.5 ml	9.5 ml
6	.6 ml	9.4 ml

Procedure for Standardization

0.5 ml of 1 µ mol/L solution of MDA standard and 0.5 ml of deionised water was taken in a test tube. To this 1 ml 0.92 mol/L of TCA was added followed by 1 ml of TBA solution. This test tube was kept in a boiling water

bath for 15 minutes. After that the tube was taken out and ice cooled. The optical density of final product was measured at 532 nm.

The same procedure was repeated with various concentrations of MDA standards. The standard graph was obtained by plotting the standard concentration in X axis and optical density in y axis.

Table

Concentration of Standard in μ mol / L	Optical density
1	.010
2	.020
3	.030
4	.040
5	.050
6	.060

Test procedure

Three test tubes were taken and marked blank, standard and test. To each test tubes the following reagents were added in order.

	Blank	Standard	Test
Standard	-	0.5 ml	-
Plasma	-	-	0.5 ml
Deionised Water	1 ml	0.5 ml	0.5 ml
TCA	1 ml	1 ml	1 ml
TBA	1 ml	1 ml	1 ml

The test tubes were kept in a boiling water bath for 15 minutes. At the end the tubes were taken out and cooled in the ice to stop the reaction. Then the contents are vortex mixtured for one minute and then centrifuged at 1000 g for 10 minutes. The clear pale supernant was collected and read at 532 nm.

Calculation

For 3 μmol/L standard : 1 ml of 3 μmol/L solution contains .003 μmol/L, from this only .5 ml was used for test.

$$\therefore \quad \frac{\text{OD.Test}}{\text{OD Standard}} \times \frac{\text{Concentration of Standard}}{\text{Volume of Standard}} \times 1000$$

$$\therefore \quad \frac{\text{OD.Test}}{\text{OD Standard}} \times \frac{0.00015}{0.5} \times 1000$$

$$\therefore \quad \frac{\text{OD.Test}}{\text{OD Standard}} \times 3$$

The results were expressed in μ mol / L.

STANDARDIZATION OF CERULOPLASMIN OXIDASE ACTIVITY BY SCHOSINKY ET AL METHOD[66]

Assay Principle

In this method O dianisidine dihydrochloride was used as substrate. This substrate was converted to yellowish brown product by ceruloplasmin oxidase and oxygen at pH 5. The pH was provided by acetate buffer. Acidification by sulfuric acid stops the enzymatic reaction and forms a purplish red solution that can be measured at 540 nm.

Reagents

Acetate buffer : - 990 ml of deionised water was taken in a 1000 ml volumetric flask. To this 13.608 gm of sodium acetate and 2.6 ml of glacial acetic acid was added and then final volume was made to 1000 ml by adding deionised water.

The pH was measured by pH meter. The final pH of the buffer was 5 and ionic strength 0.1.

Sulfuric acid: 9 mol / Ltr

125 ml of concentrated H_2So_4 was taken in measuring jar and 125ml of deionised water was added to make 250ml of 9 mol / Ltr Sulfuric acid.

O - dianisidine dihydrochloride : 7.88 mmoL/L

250 mg of O - dianisidine dihydrochloride was taken in a 100 ml volumetric flask, and deionised water was added slowly until it completely dissolved. The final volume was made to 100 ml by adding deionised water. This reagent was stored in a brown glass reagent bottle and kept at 40^0C.

Procedure

Two test tubes were taken. One test tube was marked 5 and another was marked 10. To each tube 0.75 ml of acetate buffer and 0.05 ml of plasma was pipetted. The tubes were kept in a 30^oC water bath. After five minutes 0.2 ml of O-dianosidine dihydrochloride was added and the time was noted. Exactly after 5 minutes the tube marked 5 was taken out from water bath and 2 ml of sulfuric acid reagent was added to stop the reaction. The resulting purplish red solution was read at 540nm. After 15 minutes the tube marked 15 was removed and the same procedure was repeated.

The Ceruloplasmin activity in international unit was calculated by

$$\text{U / Liter} = \frac{\text{Absorbance}}{\text{Asorptivity}} \text{ x } \frac{1}{t} \text{ x } \frac{1}{b} \text{ x } 60 \text{ x } 1000$$

$$\text{U / Liter} = \frac{\text{Absorbance}}{\text{Absorptivity}} \times \frac{1}{t} \times \frac{1}{b} \times 60 \times 1000$$

Where

$$\text{Absorbance} = A_{15} - A_5$$

A_{15} and A_5 were the measured absorbances of the "15 minutes and 5 minutes" solution.

Absorptivity = 9.6 [molar absorptivity of coloured solution in terms of substrate consumed (μ mol^{-1} cm^{-1})]

t = incubation period (10 minutes)

b = optical length (1 cm)

60 = volume correction factor (0.05 ml of plasma was incubated in 1 ml of reaction mixture which is diluted to 3 ml with 9 molar sulfuric acid)

Substituting the values in the above expression

$$\text{Activity} = \frac{\text{O.D.}\ (A_{15} - A_5)}{9.6\ \mu\ \text{mol}^{-1}\text{cm}^{-1}} \times \frac{1}{10\ \text{minutes}} \times \frac{1}{1\ \text{cm}} \times 60 \times 1000$$

Activity (u / lit) = Absorbance $\times$ 6.25×10^2

PROTEINURIA

Principle

This principle in known as protein error of indicator.

The reactive portion of the stick is coated with the buffered indicator. It changes colour in the presence of protein. In dipstick method (AlbustiX – BIOLAB diagnostics)[67] bromophenol blue buffered to pH3 with citrate, is present predominantly in the protonated yellow form. When protein is added, the affinity of the anionic form of the indicator dye for protein cause a shift of equilibrium between anionic and protonated forms of the indicator towards the formation of blue anionic species.

The intensity of the blue colour produced is proportional to the concentration of protein present in the urine.

Procedure

The yellow coloured portion of the dip stick was dipped in the urine and then allowed to dry for 30 seconds. After 30 seconds the colour change was matched with the colours given in the colour index.

Yellow - Nil

Yellowish green - 1 + ≥ 30 mg

Various shades of blue from 2 + to 4 +

1 + corresponds to 30 mg / dl

2 + = 100 mg / dl

3 + = 300 mg / dl

4+ = 1000 mg/ dl

RESULTS

A total number of 90 subjects were included in the study. They were divided into three groups.

Group 1 : Unpregnant normotensive women N = 30.

Group 2 : Uncomplicated normotensive pregnancies N = 30.

Group 3 : Preeclampsia pregnancies N = 30

The three groups were matched for age and parity.

Statistical Analysis

- In all the groups one way ANOVA was used to calculate the P valve.
- Multiple range test by Tukey - HSD procedure was employed to identify the significant groups at 5% level.
- Pearson correlation analysis was carried out to assure the relationship between MDA and CP in each study group.

Table 1

Comparison of mean levels of plasma MDA between non pregnant (group 1), un complicated pregnancy (group 2),and preeclampsia(group 3)

Group	N	MDA levels Mean ± SD in μ mol / L	p	Significant groups at 5% Level
1	30	2.5 ± 0.3	< 0.001	
2	30	3.9 ± 0.3		2 vs 1
3	30	4.7 ± 0.3		3 vs 2

The mean level of plasma MDA of group 2 was compared with the mean level of plasma MDA of group 1. The mean level of plasma MDA was significantly higher in group 2 than in group 1 ($p<0.001$)

The mean level of plasma MDA of group 3 was compared with the mean level of plasma MDA of group 2. The mean level of plasma MDA was significantly higher in group 3 than in group 2 ($p<0.001$).

Table 2

Comparison of mean levels of plasma CP activity between non pregnant (group 1), un complicated pregnancy (group 2),and preeclampsia(group 3)

Group	N	CP activity Levels Mean ± SD (U/L)	p	Significant groups at 5% Level
1	30	156.9 ± 12.6	< 0.001	
2	30	337.8 ± 26.5		2 vs 1
3	30	269.9 ± 22.8		3 vs 2

The mean level of plasma CP activity of group 2 was compared with the mean level of plasma CP activity of group 1. The mean level of plasma CP activity was significantly higher in group 2 than in group 1 ($p<0.001$)

The mean level of plasma CP activity of group 3 was compared with the mean level of plasma CP activity of group 2. The mean level of plasma CP activity was significantly lower in group 3 than in group 2 ($p<0.001$)

Table 3

Comparison of mean levels of MDA / CP ratios between non pregnant (group 1), un complicated pregnancy (group 2),and preeclampsia(group 3)

Group	N	MDA / CP ratio Mean ± SD	p	Significant groups at 5% Level
1	30	0.016 ± 0.002	< 0.001	
2	30	0.011 ± 0.001		2 vs 1
3	30	0.017 ± 0.001		3 vs 2

The mean ratio of MDA / CP of group 2 was compared with the mean ratio of MDA / CP of group 1. The mean ratio of MDA / CP in group 2 was significantly lower in group 2 than in group 1 ($p<0.001$)

The mean ratio of MDA / CP of group 3 was compared with the mean ratio of MDA / CP of group 2. The mean ratio of MDA / CP is significantly higher in group 3 than in group 2 ($p<0.001$)

Table 4

Parity wise comparison (parity 0) of mean levels of plasma MDA between non pregnant (group 1), un complicated pregnancy (group 2),and preeclampsia(group 3)

Group	N	MDA levels Mean ± SD µ mol / L	p	Significant groups at 5% Level
1	5	2.28 ± 0.19	< 0.000	
2	16	3.87 ± 0.25		2 vs 1
3	15	4.69 ± 0.28		3 vs 2

The mean level of plasma MDA of group 2 was compared with the mean level of plasma MDA of group 1. The mean level of plasma MDA was significantly higher in group 2 than in group 1 ($p<0.000$)

The mean level of plasma MDA of group 3 was compared with the mean level of plasma MDA of group 2. The mean level of plasma MDA was significantly higher in group 3 than in group 2 ($p<0.000$)

Table 5

Parity wise comparison (parity 0) of mean levels of plasma CP activity between non pregnant (group 1), un complicated pregnancy (group 2),and preeclampsia(group 3)

Group	N	CP activity Levels Mean ± SD (U/L)	p	Significant groups at 5% Level
1	5	160.25 ± 10	< 0.000	
2	16	336.90 ± 20		2 vs 1
3	15	269.49 ± 25		3 vs 2

The mean level of plasma CP activity of group 2 was compared with the mean level of plasma CP activity of group 1. The mean level of plasma CP activity was significantly higher in group 2 than in group 1 ($p<0.000$)

The mean level of plasma CP activity of group 3 was compared with the mean level of plasma CP activity of group 2. The mean level of plasma CP activity was significantly lower in group 3 than in group 2 ($p<0.000$)

Table 6

Parity wise comparison (parity 0) of mean levels of MDA / CP ratio between non pregnant (group 1), un complicated pregnancy (group 2),and preeclampsia(group 3)

Group	N	MDA / CP Ratio Mean ± SD	p	Significant groups at 5% Level
1	6	.0168 ± .003	< 0.000	
2	16	.0111 ± .0010		2 vs 1
3	15	.0161 ± .008		3 vs 2

The mean ratio of MDA / CP of group 2 was compared with the mean ratio of MDA / CP of group 1. The mean ratio of MDA / CP in group 2 was significantly lower in group 2 than in group 1 ($p<0.000$)

The mean ratio of MDA / CP of group 3 was compared with the mean ratio of MDA / CP of group 2. The mean ratio of MDA / CP is significantly higher in group 3 than in group 2 ($p<0.000$)

Table 7

Parity wise comparison (parity 1) of mean levels of plasma MDA between non pregnant (group 1), un complicated pregnancy (group 2),and preeclampsia(group 3)

Group	N	MDA levels Mean ± SD (µ mol / L)	p	Significant groups at 5% Level
1	18	2.50 ± 0.30		
2	9	3.95 ± 0.30	< 0.000	2 vs 1
3	10	4.63 ± 0.26		3 vs 2

The mean level of plasma MDA of group 2 was compared with the mean level of plasma MDA of group 1. The mean level of plasma MDA was significantly higher in group 2 than in group 1 ($p<0.000$)

The mean level of plasma MDA of group 3 was compared with the mean level of plasma MDA of group 2. The mean level of plasma MDA was significantly higher in group 3 than in group 2 ($p<0.000$)

Table 8

Parity wise comparison (parity 1) of mean levels of plasma CP activity between non pregnant (group 1), un complicated pregnancy (group 2),and preeclampsia(group 3)

Group	N	CP activity Levels Mean ± SD (U/L)	p	Significant groups at 5% Level
1	18	154.49 ± 12.72		
2	9	341.68 ± 27.53	< 0.000	2 vs 1
3	10	269.18 ± 22.7		3 vs 2

The mean level of plasma CP activity of group 2 was compared with the mean level of plasma CP activity of group 1. The mean level of plasma CP activity was significantly higher in group 2 than in group 1 ($p<0.000$)

The mean level of plasma CP activity of group 3 was compared with the mean level of plasma CP activity of group 2. The mean level of plasma CP activity was significantly lower in group 3 than in group 2 ($p<0.000$)

Table 9

Parity wise comparison (parity 1) of mean levels of MDA / CP ratio between non pregnant (group 1), un complicated pregnancy (group 2),and preeclampsia(group 3)

Group	N	MDA / CP Ratio Mean ± SD	p	Significant Groups at 5% level
1	18	.0164 ± .0023	< 0.000	
2	9	.0112 ± .0011		2 vs 1
3	10	.0168 ± .0012		3 vs 2

The mean ratio of MDA / CP of group 2 was compared with the mean ratio of MDA / CP of group 1. The mean ratio of MDA / CP in group 2 was significantly lower in group 2 than in group 1 ($p<0.000$)

The mean ratio of MDA / CP of group 3 was compared with the mean ratio of MDA / CP of group 2. The mean ratio of MDA / CP is significantly higher in group 3 than in group 2 ($p<0.000$)

Table 10

Parity wise comparison (parity 2) of mean levels of plasma MDA between non pregnant (group 1), un complicated pregnancy (group 2),and preeclampsia(group 3)

Group	N	MDA levels Mean ± SD µ mol / L	p	Significant Groups at 5% level
1	7	2.65 ± .21	< 0.000	
2	5	3.94 ± .28		2 vs 1
3	5	4.64 ± .25		3 vs 2

The mean level of plasma MDA of group 2 was compared with the mean level of plasma MDA of group 1. The mean level of plasma MDA was significantly higher in group 2 than in group 1 ($p<0.000$)

The mean level of plasma MDA of group 3 was compared with the mean level of plasma MDA of group 2. The mean level of plasma MDA was significantly higher in group 3 than group 2 ($p<0.000$)

Table 11

Parity (P2) wise Comparison of Mean Ceruloplasmin activity between Control (Group1), Uncomplicated Pregnancy (Group 2) and Preeclampsia (Group 3)

Group	N	CP activity Levels Mean ± SD (U/L)	p	Significant Groups at 5% level
1	7	160.53 ± 13.83		
2	5	333.62 ± 27.47	< 0.000	2vs1
3	5	272.25 ± 20.22		3vs2

The mean level of plasma CP activity of group 2 was compared with the mean level of plasma CP activity of group 1. The mean level of plasma CP activity was significantly higher in group 2 than in group 1 ($p<0.000$)

The mean level of plasma CP activity of group 3 was compared with the mean level of plasma CP activity of group 2. The mean level of plasma CP activity was significantly lower in group 3 than in group 2 ($p<0.000$)

Table 12

Parity wise comparison (parity 2) of mean levels of MDA / CP ratio between non pregnant (group 1), un complicated pregnancy (group 2),and preeclampsia(group 3)

Group	N	MDA /C P ratio Mean ± SD	p	Significant Groups at 5% level
1	7	.0161 ± .0018	< 0.000	
2	5	.0114 ± .0013		2 vs 1
3	5	.0166 ± .005		3 vs 2

The mean ratio of MDA / CP of group 2 was compared with the mean ratio of MDA / CP of group 1. The mean ratio of MDA / CP in group 2 was significantly lower in group 2 than in group 1 ($p<0.000$)

The mean ratio of MDA / CP of group 3 was compared with the mean ratio of MDA / CP of group 2. The mean ratio of MDA / CP is significantly higher in group 3 than in group 2 ($p<0.000$)

Table 13

Correlation between MDA levels and CP activity in plasma from control, uncomplicated pregnancy and preeclampsic group

Groups	r	p
Non Pregnant Control	0.04	0.41
Uncomplicated pregnancy	0.24	0.10
Pre eclampsia	0.77	0.0001

There was no correlation between MDA and CP in control and normal pregnant group. There was correlation between MDA and CP in preeclampsic group.($p < 0.0001$)

Table 14

Parity wise comparison of mean levels of plasma MDA in non pregnant group

(Group 1)

Parity	N	MDA levels Mean ± SD in µ mol / L	p-value
0	5	2.28 ± 0.19	0.0774
1	18	2.56 ± 0.30	
2	7	2.65 ± 0.21	

The mean levels of plasma MDA of various parities in group 1 were compared to each other [P_0 vs P_1; P_1 vs P_2; P_2 vs P_1]. No two groups were significantly different at the 0.050 level [p= 0.0774].

Table 15

Parity wise comparison of mean levels of plasma CP activity in non pregnant group
(Group 1)

Parity	N	CP activity levels Mean ± SD (U/L)	p-value
0	5	160.25 ± 10.73	0.468
1	18	154.49 ± 12.72	
2	7	160.53 ± 13.85	

The mean levels of plasma CP activity of various parities in group 1 were compared to each other [P_0 vs P_1; P_1 vs P_2; P_2 vs P_1]. No two groups were significantly different at the 0.050 level [p = 0.468].

Table 16

Parity wise comparison of mean ratios of MDA / CP in non pregnant Control group (Group 1)

Parity	N	MDA / CP ratio Mean ± SD	p-value
0	5	0.0168 ± 0.001	0.0632
1	18	0.0164 ± 0.002	
2	7	0.0161 ± 0.001	

The mean ratio of MDA / CP ratios of various parities of group 1 were compared to each other [P_0 vs P_1; P_1 vs P_2; P_2 vs P_1]. No two groups were significantly different at the 0.050 level [p = 0.0632].

Table 17

Parity wise comparison of mean levels of plasma MDA in un complicated pregnancies group (Group 2)

Parity	N	MDA levels Mean ± SD in µ mol / L	p-value
0	16	3.91 ± 0.26	0.769
1	9	3.95 ± 0.30	
2	5	3.94 ± 0.28	

The mean levels of plasma MDA of various parities in group 2 were compared to each other [P_0 vs P_1; P_1 vs P_2; P_2 vs P_1]. No two groups were significantly different at the 0.050 level [p = 0.769].

Table 18

Parity wise comparison of mean levels of plasma CP activity in un complicated pregnancies

(Group 2)

Parity	N	CP activity levels Mean ± SD (U/L)	p-value
0	16	336.99 ± 27.11	0.855
1	9	341.68 ± 27.53	
2	5	333.62 ± 27.47	

The mean levels of plasma CP activity of various parities in group 2 were compared to each other [P_0 vs P_1; P_1 vs P_2; P_2 vs P_1]. No two groups were significantly different at the 0.050 level [p = 0.855].

Table 19

Parity wise comparison of mean ratios of MDA / CP in un complicated pregnancies

(Group 2)

Parity	N	MDA / CP ratio Mean ± SD	p-value
0	16	0.0111 ± 0.0010	0.818
1	9	0.0112 ± 0.0011	
2	5	0.0114 ± 0.0013	

The mean ratio of MDA / CP ratios of various parities of group 2 were compared to each other [P_0 vs P_1; P_1 vs P_2; P_2 vs P_1]. No two groups were significantly different at the 0.050 level [p = 0.818].

Table 20

Parity wise comparison of mean levels of plasma MDA in preeclampsic pregnancies (Group 3)

Parity	N	MDA levels Mean ± SD in µ mol / L	p-value
0	15	4.69 ± 0.28	0.849
1	10	4.63 ± 0.26	
2	5	4.64 ± 0.25	

The mean levels of plasma MDA of various parities in group 3 were compared to each other [P_0 vs P_1; P_1 vs P_2; P_2 vs P_1]. No two groups were significantly different at the 0.050 level [p = 0.849].

Table 21

Parity wise comparison of mean levels of plasma CP activity in preeclampsic group (Group 3)

Parity	N	CP activity levels Mean ± SD (U/L)	p-value
0	15	269.49 ± 25.01	0.969
1	10	269.18 ± 22.72	
2	5	272.25 ± 20.22	

The mean levels of plasma CP activity of various parities in group 3 were compared to each other [P_0 vs P_1; P_1 vs P_2; P_2 vs P_1]. No two groups were significantly different at the 0.050 level [p = 0.969].

Table 22

Parity wise comparison of mean ratios of MDA / CP in preeclampisic pregnancies

(Group 3)

Parity	N	MDA / CP ratio Mean ± SD	p-value
0	15	0.0160 ± 0.0008	0.583
1	10	0.0168 ± 0.0012	
2	5	0.0166 ± 0.0005	

The mean ratio of MDA / CP of various parities of group 3 were compared to each other [P_0 vs P_1; P_1 vs P_2; P_2 vs P_1]. No two groups were significantly different at the 0.050 level [p = 0.583].

DISCUSSION

The etiology and pathogenesis of pregnancy syndrome preeclampsia remain poorly understood. The diverse manifestation of preeclampsia including altered vascular reactivity, vasospasm are derived from pathological changes within the maternal vascular endothelium.

The present study was designed to find out the role of oxidative stress in pathogenesis of preeclampsia. Oxidative stress is an imbalance favouring oxidant over antioxidant system.

Hubel, C.A. reported that lipid peroxidation products may mediate disturbance of maternal vascular endothelium. He also reported decreased levels of ceruloplasmin in preeclampsic pregnancies.

Atamer, et al evaluated the plasma MDA and CP level in 25 non pregnant and 25 healthy pregnant women and reported the increased level of MDA and CP in pregnant women compared to non pregnant women. ($p<0.001$)[68]

In this study increased MDA and CP activity were found in uncomplicated normotensive pregnancy group compared to normotensive non pregnant control group ($p < 0.001$).

The ratio of MDA/ CP was decreased in normotensive pregnancy group compared to normotensive nonpregnant group ($p < 0.001$). This indicates that oxidative stress does not occur in group 2. Results obtained indicate increased ceruloplasmin level of maternal plasma is possibly due to a compensatory

response to increased lipid peroxidation in normal pregnancy. This antioxidant defence protects the cells against oxidative damage.

Akshoy, H et al., evaluated MDA and ceruloplamin level in 21 normotensive pregnancies and 21 preeclampsic pregnancies. They reported higher level of MDA and lower level of ceruloplasmin activity in preeclampsic pregnancies compared to normotensive pregnancies[69].

The present study shows increased MDA levels and decreased CP activity in preeclampsic pregancies compared to normotensive pregnancies ($p < 0.001$)

Increased MDA/ CP ratio was observed in preeclampsic group compared to normotensive pregnancies.($p<0.001$)

This increased MDA/ CP ratio ($p<0.001$) in preeclampsic group implies an impaired oxidant and antioxidant balance in favour of oxidants and can be defined as oxidative stress.

H. Gurer Orhan et al investigated 12 normotensive non pregnant control, 12 uncomplicated normotensive pregnancies and 7 preeclampsic pregnancies. They observed correlation between MDA and CP activity in preeclampsic group[70].

In the present study no correlation was observed between MDA & CP in group 1 and group 2. There was correlation between MDA and CP in preeclampsic group ($p < 0.001$) which proves the presence of protection against oxidative stress.

CONCLUSION

Hydroxyl and alkoxyl radicals are generated during lipid peroxidation. MDA, HNE and Isoprostanes are produced during the decomposition of lipid hydroperoxides. All these products are probably the cause of the pathogenesis of damage to vascular endothelium.

The increased prooxidant activity is augmented by decreased levels of many intra and extra cellular antioxidants. Such a role of oxidative stress as a factor for pathogenesis of preeclampsia can be postulated as there are reports of decreased level of many anti-oxidants like superoxide dismutase, glutathione, vitamin C, vitamin E and ceruloplasmin in preeclampsia.

The present study has found an increased oxidative stress as shown by increased plasma MDA, decreased plasma CP activity and increased MDA / CP ratio in patient having preeclampsia.

Oxidative stress may be the point at which multiple factors converge resulting in endothelial dysfunction and consequent clinical manifestation of preeclampsia. From this study it is concluded that oxidative stress is one of the major factors in the pathogenesis of preeclampsia.

BIBLIOGRAPHY

1. Gupta, S et al. The role of oxidative stress and lipid peroxidation in preeclampsia. Obstet and Gynecol Surv. 60 (12); 807 - 16, 2005.

2. Roes, EM et al. Deficient detoxyfying capacity in the pathophysiology of preeclampsia. Med. Hypoth. 55; 415 - 418, 2000.

3. Carl. A. Hubel. Oxidative stress in the pathogenesis of preeclampsia. Proc Soc Exper Biol Med. 222; 222 - 235, 1999.

4. Atamer, Y et al. Lipid peroxidation, antioxidant defence, status of trace metals and leptin levels in preeclampsia. Eur J Obstet Gynec Reprod Biol. 3(1); 119(1); 60 - 66, 2005.

5. Gutterridge, JMC et al. Ceruloplasmin physiological and pathological perspectives. Crit Rev Clin Lab Sci 14 : 257, 1981.

6. Gurer, H et al. Correlation between MDA and CP activity in preeclampsia. J Clinical Biochemistry. 34; 505 - 506, 2000.

7. Southern, PA et al. Free radical in medicine and human disease. Mayo Clin Proc. 63; 390, 1998.

8. Seis, H. Oxidative stress II. Oxidants and antioxidants. Academic press. London. Page 396, 1991.

9. Roberts, JM et al. Preeclampsia : An endothelial cell disorder. Am J Obst Gynec. 163; 1365 - 66, 1999.

10. Walsh, SC et al. Lipid peroxidation in pregnancy. Hypertension pregnancy. 13; 1 - 25, 1994.

11. Sen, CK et al. Anti oxidant and redox regulation of gene transcription. FASEB J. 10; 709 - 720, 1996.

12. Halliwell, B and Gutteridge, JMC. The anti oxidants of human extracellular fluids. Arch Biochm Biophys 280: 1; 1990.

13. Hypertensive disorders in pregnancy. Williams Obstetrics. 21st edition 2003. Editor : F Gary Cunningham Pub : Mcgraw & Hill, Page :

14. Conde - Agudelo A. Risk factors for preeclampsia. Br J Obstet Gynecol. 107 : 205, 2000.

15. Chesley LC. Diagnosis of preeclampsia. J Osbst Gynecol. 64 : 423, 1985.

16. Hypertensive disorders in pregnancy. Williams Obstetrics. 21st edition 2003. Editor : F Gary Cunningham Pub : Mcgraw & Hill, Page : 569.

17. Hypertensive disorders in pregnancy. Williams Obstetrics. 21st edition 2003. Editor : F Gary Cunningham Pub : Mcgraw & Hill, Page : 517.

18. Benedetti, TJ et al. Hemodynamic observations in severe preeclampsia. Am J Obst Gynecol 136 : 465, 1980.

19. Pritchard JA. Hemotological abnormalities associated with severe toxemic pregnancy. N Eng J Med. 250 : 87, 1954.

20. Weir RJ. Plasma renin and aldosterone in hypertensive pregnancy. Lancet 1 : 291, 1971.

21. Spargo, B. Glomerular capillary endotheliosis in toxemia of pregnancy. Arch Pathol. 68 : 593; 1959.

22. Taufield, PA. Hypocalciuria in preeclampsia. N Eng J Med. 316 : 715, 1987.

23. Barton, JR et al. Elevated liver enzymes in HELLP syndrome. Am J Obstet Gynecol. 167 : 1538, 1992.

24. Rodman CW. Preeclampsia and the placenta. 12 : 301 - 308, 1991.

25. Cross JC. Trophoblastic function in normal and preeclamptic pregnancy. Fetal Matern Med Rev. 8 : 57 - 66, 1996.

26. De Wolf, F et al. The human placental bed. Am J Obstet Gynecol. 137 : 58, 1980.

27. Landesman, R et al. The bulbar conjuctival vascular bed in the toxemias of pregnancy. Am J obstet Gynecol 68 : 170, 1954.

28. Browne FJ. Sensitization of the vascular system in preeclampsic toxemia and eclampsia. Br J Obstet Gynecol. 53 : 510, 1946.

29. Walsh, SW. Preeclampsia : An imbalance in placental prostacyclin and thromboxane production. Am J Obst Gynecol, 152 : 335, 1985.

30. Baker, PN et al. Elevated serum level of VEGF in patient with preeclampsia. Obstet Gynecol. 86 : 15, 1995.

31. Lorentzen, B et al. Plasma lipids and vasular dysfunction in preeclampsia, Sem Reprod Endocrinol. 16 : 33 - 39, 1998.

32. Lorentzen, B et al. Fatty acid patterns in sera of women with normal and pereclampsic pregnancy. Br J Gynecol. 102 : 530 - 537, 1995.

33. Chait, A. Susceptibility of small dense LDL to oxidation. Am J Med. 94 : 350 - 336, 1993.

34. Bonet, B et al. Metabolism of modified LDL by cultured human placental cells. Alberosclerosis. 112 : 125 - 136, 1995.

35. Esterbauer, H. Cytotoxicity and genotoxicity of lipid peroxidation products. Am J Clin Nutri. 57 : 779 - 786, 1993.

36. Barry Halliwell, John M.C. Gutteridge. Free radical in biology and medicine. 3rd Edition 1999 Oxford Science Publiation. Page 23.

37. Barber, DJW et al. Reaction of radicals with lecithin bilayer. Radiat Res. 74 : 51, 1978.

38. Cellular injury and adaptation. Robbins pathological basis of disease. 4th edition 1989. Editors : Robbins, Kumar, Cotran, Publisher: W.B.Saunders page 10.

40. Halliwell, B and Gutteridge JMC. Role of catalytic metal ions in human disease. Methods Enzymol, 1 : 186, 1990.

41. Gutteridge, JMC. Ceruloplasmin : Physiological and pathological perspectives. Crit Rev Clin Lab Sci. 14 : 257, 1981.

42. Frank, H et al. Mass spectrometric detection of cross linked FFA formed during radical induced lesion of lipid membrane. Biochem J 260 : 873, 1989.

43. Gardner, H.W. Oxygen radical chemistry of PUFAS. Free Rad Biol Med. 7 : 65, 1989.

44. Mechanism of damage of cellular targets by oxidative stress. Free Rad Biol Med. 3rd edition 1998. Editors: Barry Halliwell and John M.C.Gutteridge. Publishers: Oxford Science Publication, page : 295.

45. Esterbauer, H et al. Chemistry and biochemistry of HNE, MDA. Free Rad Biol Med. 11 : 81, 1991.

46. Benamira, M et al. Induction of mutation by replication of MDA modified DNA carcinogenesis. 16 : 93, 1995.

47. Muller, K et al. Cytotoxic and chemotactic potencies of several aldehyde components of oxidized LDL. FEBS Lit. 388 : 165, 1996.

48. Levin, RL et al. Oxidative stress and defense. Proc Natl Acad Sci USA. 93 : 15036 - 40, 1997.

49. Davies KJA. Oxidative stress and defense. J Biol Chem. 262 : 9895-901, 1987.

50. Dupart, F et al. Susceptibility of cloned K^+ channels to ROS. Proct Natl. Acad. 92 : 11796, 1995.

51. Roberts, LJ et al. The generation and actions of Isoprostanes. Biochem Biophys Acta. 1271 : 61, 1997.

52. Esterbauer, H et al. Chemistry and biochemistry of MDA, Free Rad Biol Med. 11 : 81, 1991.

53. Barry Halliwell, Jhon. M.C. Gutteridge. Free radicals in Biology and medicine. 3rd edition oxford science publication. 1999, page 302.

54. Barry Halliwell, John. M.C. Gutteridge. Antioxidant defences. Free Rad Biol Med 3rd edition. Oxford science publication 1999, page 105.

55. Meister, A. Mitrochondrial changes associated with glutathione deficiency. Biochem Bio Phys Acta 1271: 35; 1995.

56. Nishikmi, M et al. Biochemistry and molecular biology of ascorbic acid biosynthesis. 25 : 17, 1996.

57. Bendich, A et al. The antioxidant role of Vitamin C. Adv. Free Rad Biol Med. 2 : 149, 1986.

58. Machlin, LJ et al. The antioxidant role of Vitamin C. Adv Free Rad Biol Med. 2 : 419 - 44, 1986.

59. Niki, M et al. Nutrition in integrated biological system. Ann NY Acad Sci. 570 : 23 - 31, 1989.

60. Maret. G. Traber. Vitamin E. Modern nutrition in health and disease. 9th edition Editor : Maurice E shills. Publisher Williams & Wilkins page : 348 - 349, 1999.

61. Maret G. Traber. Vitamin E. Modern nutrition in health and disease. 9th edition. Editor: Maurice E shills. Publishers: Williams & Wilkins page : 350, 1999.

62. James. A. Thomas. Oxidative stress and oxidant defence : Modern nutrition in health and disease. 9th edition. Editor : Maurice E Shills. Publishers : Williams & Walkins, page : 758, 1999.

63. Paulik M.D, Weiss ML, Ceruloplasmin. In: The Plasma proteins. Vol. II, 2nd Ed. F. Putman Ed : New York Academic Press, 1975 Page : 52-109.

64. Gutteridge, JMC and Stocks, J. Ceruloplasmin physiological and pathological perspectives. Crit. Rev. Clin. Lab. Sci. 14 : 257, 1981.

65. Beuje JA, Avst JD et al. Thiobarbituric acid assay for lipid peroxides. Methods in enzymology, 52 : 302 - 310, 1978.

66. Schonisky, KH et al. Measurement of C.P from its oxidase activity by use of O-dianisidine dihydrochloride. Clin Chem. 20 : 1550 - 63, 1974.

67. Proteins and Amino acids. Teitz fundamentals of clinical chemistry. Editor : Morbert. W.Tietz. Publisher : W.B.Saunders. 2nd edition, page : 359, 1982.

68. Atamer, Y et al. Lipid peroxidation antioxidant levels in pre eclampsia. Eur. J. Obst Gynecol, 1 : 60 - 66, 2005.

69. Akshoy et al. Antioxidant potentials in women with pre eclampsia. Investig Med. 51(5) 284 - 7, 2003.

70. Gurer, H et al. Correlation between MDA and CP activity in preeclampsia. J. Clin. Biochem. 34 : 505 - 506, 2000.

LIST OF ABBREVIATIONS USED

MDA	:	Malondialdehyde
CP	:	Ceruloplasmin
BP	:	Blood Pressure
mmHg	:	Millimeter mercury
LDH	:	Lactate dehydrogenase
GFR	:	Glomerular filtration rate
FFA	:	Free fatty acid
TGL	:	Triglycerides
LDL	:	Low density lipoprotein
HDL	:	High density lipoprotein
$OH^{\bullet}$	:	Hydroxyl radical
$RO_2^{\bullet}$	:	Peroxyl rdical
$RO^{\bullet}$	:	Alkoxyl radical
$O_2^{\bullet -}$	:	Superoxide radical
Fe^{2+}	:	Ferrous ion
Fe^{3+}	:	Ferric ion
H_2O_2	:	Hydrogen peroxide
ROOH	:	Lipid hydroperoxide
PUFA	:	Polyunsaturated Fatty acid
$R^{\bullet}$	:	Carbon centered radical
Cu^{+}	:	Cuprous ion
CU^{2+}	:	Cupric ion
DNA	:	De oxy ribo nucleic acid

GS – SG	:	Oxidized Glutathione
GSH	:	Reduced glutathione
NADPH	:	Nicotinamide adenine di nucleotide phosphate reduced
NADP	:	Nicotinamide adenine di nucleotide phosphate
HMP	:	Hexose Mono Phosphate
NADH	:	Nicotinamide adenine di nucleotide reduced
NAD	:	Nicotinamide adenine di nucleotide
Vit. E - $O^{\bullet}$	:	Tocopheroxyl rdical
EDTA	:	Ethylene diamine tetra acetic acid
TBA	:	Thiobarbituric acid
TCA	:	Trichloroacetic acid
Hcl	:	Hydrochloric acid
O.D.	:	Optical density
H_2SO_4	:	Sulfuric acid
μmol	:	Micromole
mmol	:	millimole
U/L	:	Units per litre

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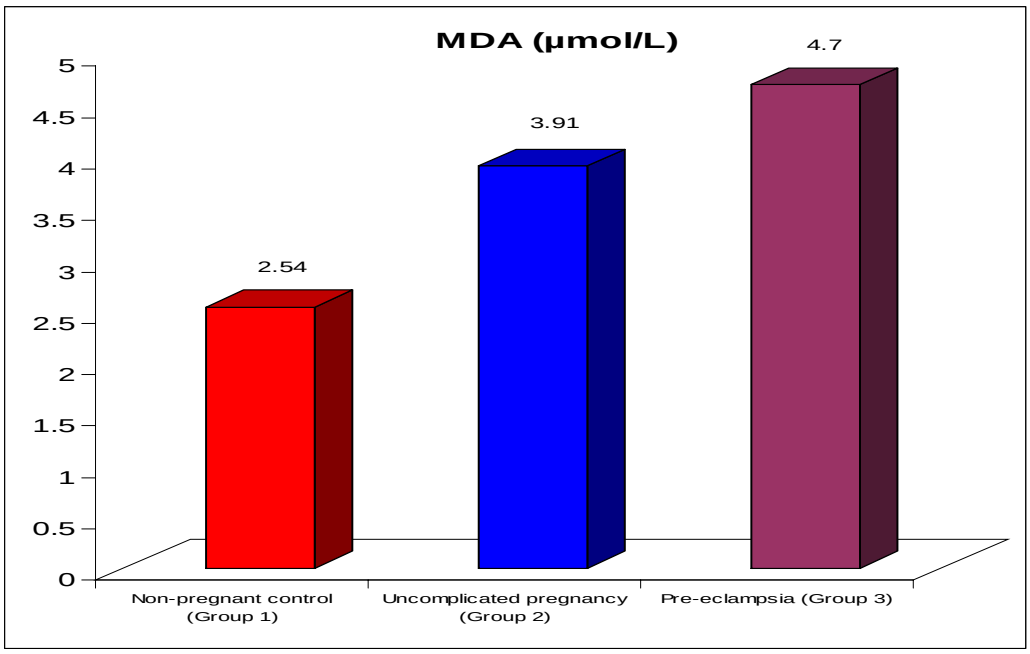
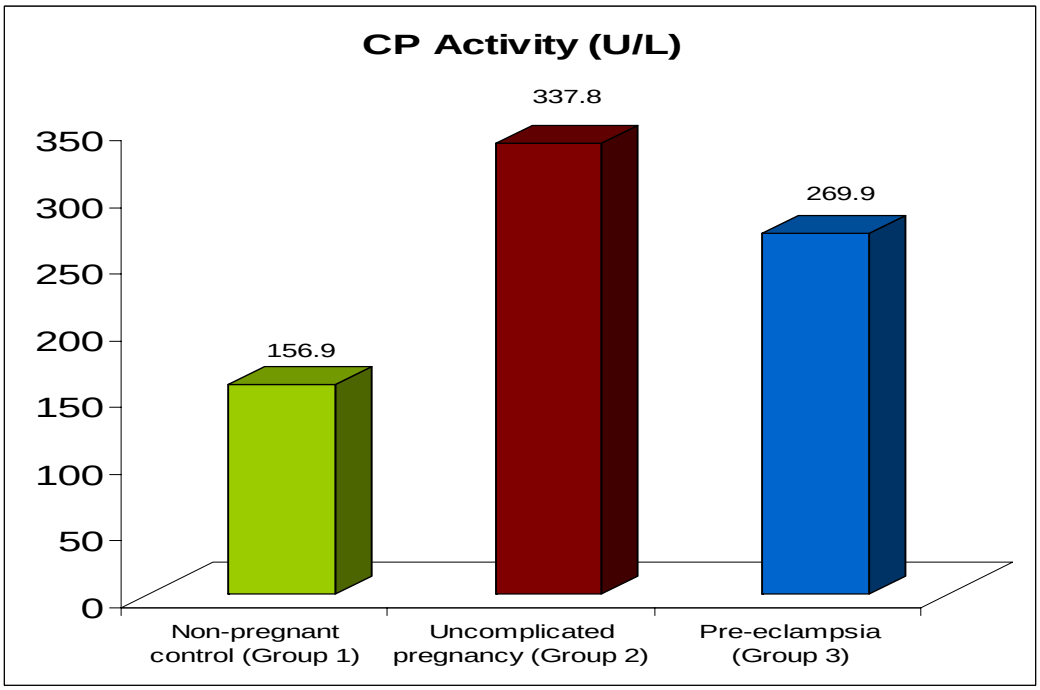
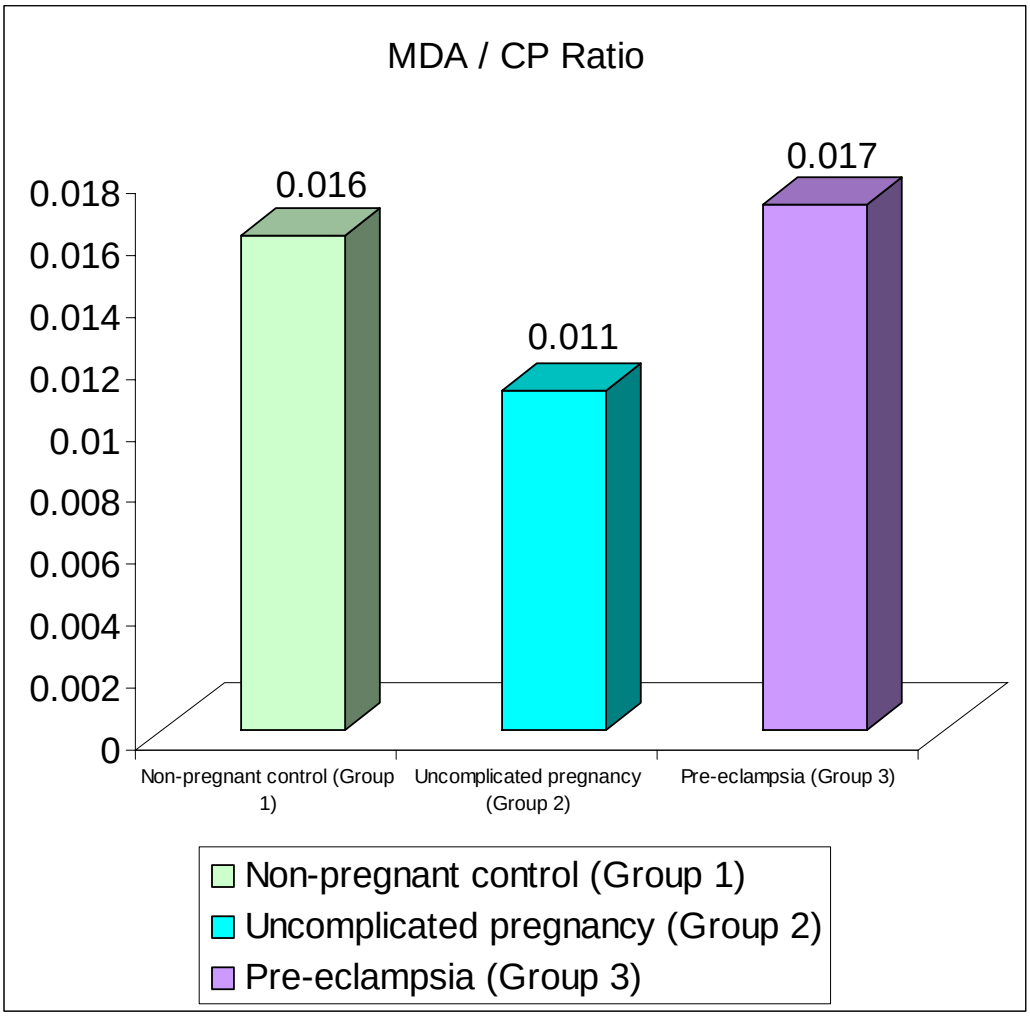


The mean level of plasma MDA is found to increase in preeclampsia (Group 3) when compared to uncomplicated pregnancy (Group 2), showing the presence of more oxidative stress in preeclampsia.



Group 2 shows increased mean level of plasma ceruloplasmin activity proving the protective function of ceruloplasmin on oxidative stress.


MDA / CP Ratio
0.018
0.016
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
0.016
0.011
0.017
Non-pregnant control (Group 1)
Uncomplicated pregnancy (Group 2)
Pre-eclampsia (Group 3)
Non-pregnant control (Group 1)
Uncomplicated pregnancy (Group 2)
Pre-eclampsia (Group 3)

PROFORMA

GROUP 1

Name : Age : Marital Status :

Parity :

Past History : Hypertension

Diabetes Mellitus

Tuberculosis

Other Systemic Disorders

General Examination : BP :

PR :

CVS :

RS :

Investigations : Urine for Albumin

M.D.A.:

C.P. :

GROUP 2 & 3 :

Name : Age :

IP / OP NO. :

Obstetric formula: Gravida / Para Gestational age :

Present history : LMP

EDD

Past History : Hypertension

Diabetes Mellitus

Tuberculosis

Infection

Other systemic disorders

General Examination : BP : PR:

CVS : RS :

INVESTIGATIONS :

Haemoglobin

Urine for albumin

M.D.A.

C.P.